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Award Number: W81XWH-07-1-0636

TITLE: Silver Foam Technologies Healing Research Program

PRINCIPAL INVESTIGATOR: Michael F. Moore, M.D.

CONTRACTING ORGANIZATION: Noble Biomaterials

REPORT DATE: September 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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				5b. GRANT NUMBER W81XWH-07-1-0636	
				5c. PROGRAM ELEMENT NUMBER	
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1. Introduction:

Blunt traumatic wounds pose a unique problem to the treating surgeon in the fact that the mechanism of the injury may be known, but the extent and degree of the injury is difficult to assess regarding the viability of the injured tissue. The “area of necrosis” may also increase because of tissue hypoxia from continuing blood loss or the effects of septic shock. Standard surgical care for such wounds focuses on surgically removing any non-viable tissue, controlling blood loss and removing any foreign material that could harbor and facilitate bacterial colonization and growth. Because many of these wounds cannot be closed primarily, the surgeon is left with packing the wound open using standard gauze dressings. The goal of this research was to produce a bandage that would interact with the wound and provide adsorptive properties greater than gauze, broad spectrum antimicrobial coverage over an extended period of time and capable of initiating and facilitating hemostasis.

2. Body:

Task 1: Research open-end hydrophilic foam:

The result of this research has produced an open-end hydrophilic foam with an absorption ratio of fifteen grams of fluid for every gram of foam. It has a pore size of fifty pores for every inch of foam. The foam's construct and structure is such that it is capable of being cut without compromise to its integrity.¹ This quality has led to the production of three bandages that are similar in size to standard gauze bandages in clinical use. Two inches in width and length and one quarter inch thick, four inches in width and length and one quarter inch thick and ten inches in length, eight inches in width and one half inch thick.

Task 2: Metalized open-end hydrophilic foam:

Uniform distribution of elemental silver throughout the foam was accomplished by the mixing of silver metalized glass beads at the fluid stage. Varying concentrations of silver glass beads were tested and all exhibited uniform distribution in the hydrophilic foam. The concentration of silver exhibiting the greatest hemostatic properties produced foam construct that contained 26.5% silver glass beads uniformly distributed throughout the open-end foam. (Appendix A)

Task 3: Identify and incorporate topical hemostatic agents:

Multiple hemostatic agents were investigated for incorporation into the foam. Of the agents that were identified and selected only two Zeolite and Silver Glass Beads agents were capable of being incorporated into the foam at sufficient concentrations without congealing. Open-ended hydrophilic foam sponges incorporating Zeolite and Silver Glass Beads were made and tested for hemostasis on human heparinized blood. The findings showed that increasing concentrations of silver glass beads enhance the ability of the sponge to absorb and coagulate blood. Zeolite did not exhibit the same hemostatic properties seen in the silver glass bead model.¹ Bandage lots were then produced and submitted for antimicrobial evaluation and biocompatibility testing.

Task 4: Establishing the antimicrobial activity of the hydrophilic foam:

Samples of the antimicrobial hemostatic foam bandages were sent to NAMSA test facility in Irvine California to test the antimicrobial effect against multiple bacteria over a seventy-two hour period. Similar samples were also submitted to Hosier Technology Center Hickory, North Carolina to evaluate the effect the foam's antimicrobial effect on MRSA. Concentrations of 10^5 to the fifth power colony forming units were employed. The findings revealed a greater than 99.9% reduction in MRSA colony forming units at twenty-four hours which was sustained to seventy-two hours. (Appendix B) Bacterial organisms tested by NAMSA showed a greater than 99% reduction of colony forming units at twenty-four hours except for *Serratia marcescens* which showed a 98% reduction in colony forming organisms in one sample. The reduction seen at the twenty-four hour time period was again sustained for seventy-two hours as reported by Biosan Laboratories. (Appendix C) Subsequent sterilization using gamma radiation by STERIS Corporation revealed retention of the antimicrobial pattern seen at twenty-four and seventy-two hours.

Task 5: Determine the safety profile of silver hydrophilic foam:

Samples of the antimicrobial hemostatic foam were submitted to NAMSA Test Facility Northwood, Ohio for cytotoxic studies. Findings showed slight to mild effects or grade two or less consistent with biocompatibility. (Appendix D). Testing in mouse and guinea pig models likewise fail to reveal any toxic effects on the animals tested. (Appendix E) Analysis by STERIS Corporation of the unsterilized foam bandage did not produce any bacterial growth. Sterilization and validation of the sustained hemostatic properties of the bandage has been performed

Task 6: Assess the logistic cost of using the silver foam bandage in clinical settings:

The protocol developed for the clinical evaluation of the silver foam bandage addresses the blunt traumatic wound that cannot be closed by direct primary closure. Various trauma centers were evaluated as to their ability and willingness to participate in clinical trials. Review of the protocol (Appendix E) was undertaken and the Community Medical Center, a level II trauma center, in Scranton, Pennsylvania and Albany Medical Center, a level I trauma center, in Albany New York. Both trauma centers agreed to participate in the clinical evaluation. Michael F. Moore, MD will act as the principal investigator at the Community Medical Center and Carl Rosati will be the principal investigator at Albany Medical Center. Submission of the protocol to the Scranton Temple Resident Program Institutional Review Board for Community Medical Center has occurred. Approval for Albany Medical Center and the US Army is in progress. The incorporation of the bandages into individual packages consistent with clinical use has occurred. Distribution will occur when sterilization has been completed and IRB approval has been obtained.

3. Key Research Accomplishments:

- Silver glass beads exhibiting a hemostatic and antimicrobial effect have been incorporated into medical grade hydrophilic foam.

- The metalized hydrophilic foam is capable of absorbing and clotting heparinized human blood.
- The antimicrobial pattern shows that it is effective against both gram positive and negative bacteria.
- The antimicrobial effect is sustained for seventy-two hours.
- The hemostatic antimicrobial foam has been able to be produce in sizes compatible with existing bandage types for clinical use.
- Sterilization of the foam bandage has occurred without changing its hemostatic or antimicrobial effect.

4. Reportable Outcomes:

- a. Quarterly reports have been submitted to the US Army attesting to the serial progress made regarding this research.
- b. Patent application has been submitted Number 12/554,727 to the United States patent office.
- c. Submission of the clinical protocol to the Scranton Residency Temple Residency Institutional Review Board has occurred.

5. Conclusions:

The completed research to date has resulted in the creation of an interactive hydrophilic foam bandage that has both hemostatic and antimicrobial attributes and is capable of absorbing fifteen grams of fluid per gram of foam. The bandage has sustained antimicrobial effects allowing it to be left in place and is capable of sterilization allowing it to be used in clinical settings.

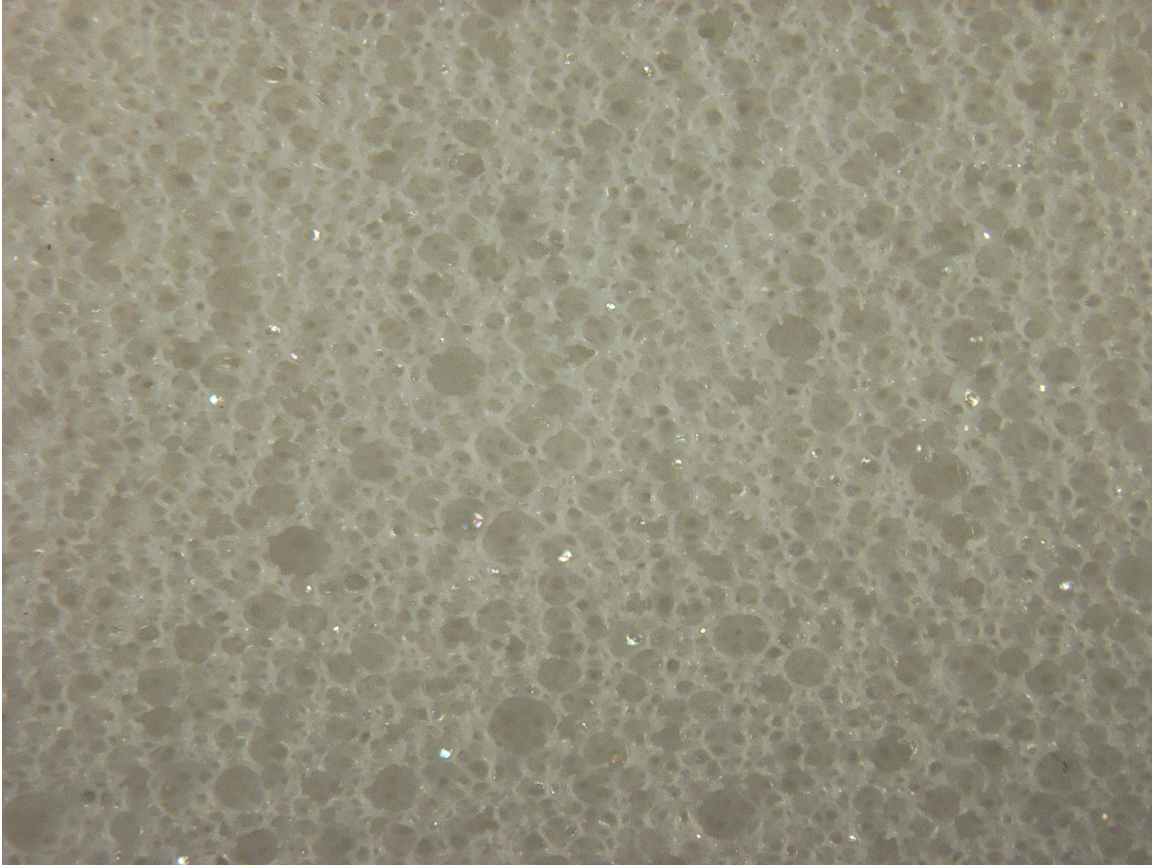
6. References:

1. 2008 Annual Report US Army Grant W81XWH-07-1-0636

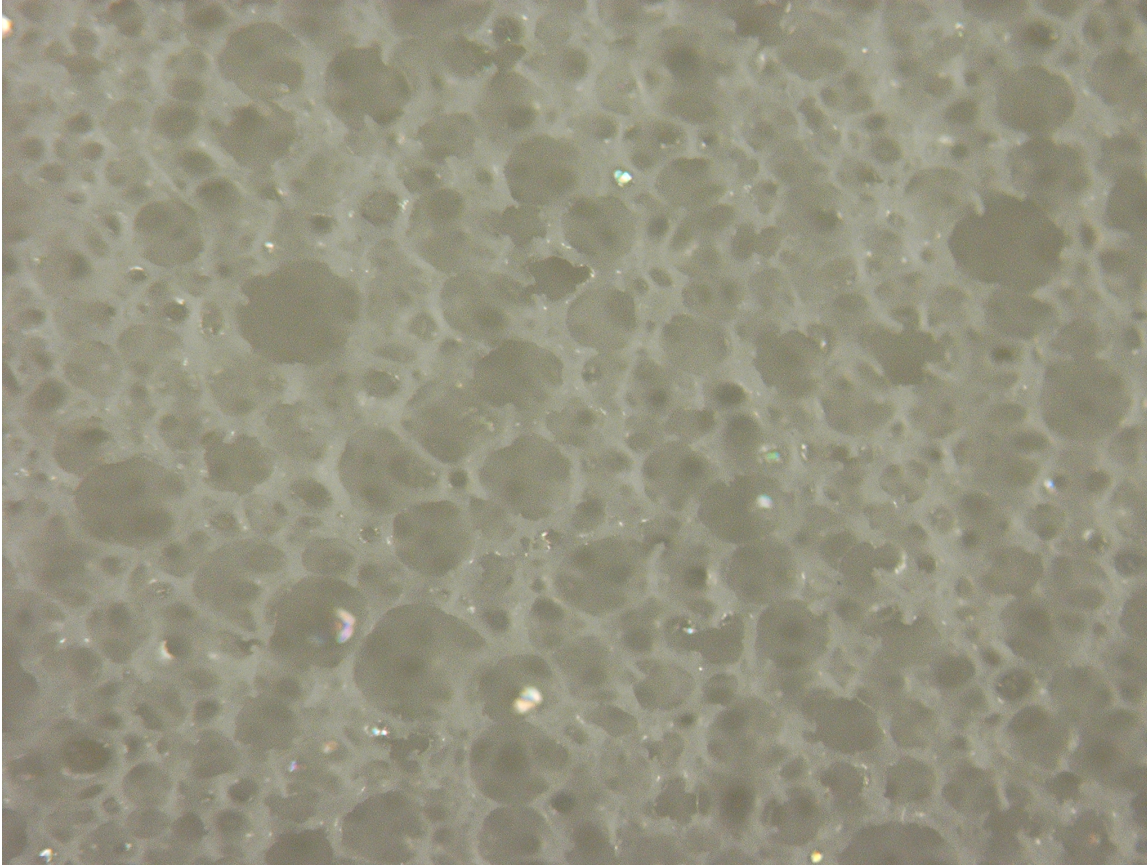
7. Appendices:

Appendix A:

Hemostatic foam-10x magnification-Pic (2).JPG



Hemostatic foam-60x magnification-Pic (1).JPG



Appendix B:

HOSIERY TECHNOLOGY CENTER

2550 Highway 70 SE
Hickory, NC 28602
(828) 327-7000 ext. 4115

Attn.:

Vinesh Naik, Ana C. De Los Santos



Noble Biomaterials
300 Palm Street
Scranton, PA 18505

Phone:

570-207-6110

REFERENCE:

TS # 35619

tested original state

SUBMITTED DATE:

12/11/08

COMPLETED DATE:

12/22/08

TEST METHOD: DETERMINING ANTIMICROBIAL ACTIVITY OF IMMOBILIZED ANTIMICROBIAL AGENTS (ASTM E 2149)

Test Article: Style: anti microbial hemostatic foam
Color: white
Other: 18 gm

Test Organism: S. aureus methicillin resistant ATCC 33591

Sample size: 1.0 g
Target inoculum Level: $(1.0-2.0) \times 10^5$ CFU/ml
wetting agent: none

Sterilization: autoclave

TS # 35619
S. aureus methicillin resistant ATCC 33591

Results CFU/sample		
Zero Contact Time	24 hr Contact Time	Percent Reduction
1.76E+05	9.90E+01	99.94%
Zero Contact Time	48 hr Contact Time	Percent Reduction
1.76E+05	9.90E+01	99.94%
Zero Contact Time	72 hr Contact Time	Percent Reduction
1.76E+05	9.90E+01	99.94%

NR = No Reduction

* Plates were spirally prepared and counted using automatic scanning.
* Dilution of organism prepared in Phosphate buffer

Maria Curry
Environmental Laboratory Manager

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Environmental Lab
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(828) 327-7000 ext. 4115

Attn.: Vinesh Naik, Ana C. De Los Santos



Noble Biomaterials
300 Palm Street
Scranton, PA 18505

Phone: 570-207-6110

REFERENCE: **TS # 36030** tested original state **SUBMITTED DATE:** 01/13/09
COMPLETED DATE: 01/16/09

TEST METHOD: DETERMINING ANTIMICROBIAL ACTIVITY OF IMMOBILIZED ANTIMICROBIAL AGENTS (ASTM E 2149)

Test Article: Style: anti microbial hemostatic foam-54
Color: white
Other:

Test Organism: S. aureus methicillin resistant ATCC 33591

Sample size: 1.0 g
Target inoculum Level: $(1.0-2.0) \times 10^5$ CFU/ml
wetting agent: none

Sterilization: autoclave

TS # 36030
S. aureus methicillin resistant ATCC 33591

Results CFU/sample		
Zero Contact Time	24 hr Contact Time	Percent Reduction
3.04E+05	9.90E+01	99.97%
Zero Contact Time	48 hr Contact Time	Percent Reduction
3.04E+05	9.90E+01	99.97%
Zero Contact Time	72 hr Contact Time	Percent Reduction
3.04E+05	9.90E+01	99.97%

NR = No Reduction

* Plates were spirally prepared and counted using automatic scanning.
* Dilution of organism prepared in Phosphate buffer

Maria Curry
Environmental Laboratory Manager

www.hosetech.com

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Hosiery Technology Center –
Environmental Lab
(828) 327-7000 ext. 4521
enviroqueen@legsource.com

Appendix C:



CONFIDENTIAL REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
9 Morgan
Irvine, CA 92618
949.951.3110

Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 2			
Antimicrobial Hemostatic Foam-54 – AB 19606	2.19 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – AB 19606	2.22 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – BA 9372	1.89 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – BA 9372	1.76 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – BD 19416	2.02 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – BD 19416	1.90 x 10 ⁵	2.82 x 10 ⁶	No Reduction
Antimicrobial Hemostatic Foam-54 – CD 13812	2.05 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – CD 13812	1.80 x 10 ⁵	1.81 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – VRE 51575	2.07 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – VRE 51575	2.22 x 10 ⁵	1.63 x 10 ⁶	No Reduction
Antimicrobial Hemostatic Foam-54 – EC 8739	1.95 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – EC 8739	1.96 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – EC 25922	1.92 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – EC 25922	1.74 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – KP 4352	2.61 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – KP 4352	1.84 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – ML 49732	2.03 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – ML 49732	1.85 x 10 ⁵	2.43 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – PV 6380	2.04 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – PV 6380	1.74 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – PM 12453	1.97 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – PM 12453	1.92 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction

Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 2			
Antimicrobial Hemostatic Foam-54 – PA 9027	2.48 x 10 ⁵	1.35 x 10 ²	99.95
CONTROL – PA 9027	1.95 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – PA 15422	2.59 x 10 ⁵	7.75 x 10 ²	99.70
CONTROL – PA 15422	2.12 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SC 10708	1.73 x 10 ⁵	7.60 x 10 ²	99.56
CONTROL – SC 10708	2.04 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SM 8100	2.21 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SM 8100	1.89 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SM 14756	2.11 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SM 14756	2.04 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SA 6538	2.10 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SA 6538	2.38 x 10 ⁵	3.30 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – SA 25923	2.45 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SA 25923	2.58 x 10 ⁵	3.80 x 10 ⁵	No Reduction

P.O. No.:
P81518

Lab Number:
09C_20640_01 / 09C_20640_02
09C_20640_03 / 09C_20640_04

MG120_000, MG120_001/S

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Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 1			
Antimicrobial Hemostatic Foam-54 – EC 25922	2.20 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – EC 25922	1.96 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – KP 4352	1.71 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – KP 4352	1.98 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – ML 49732	2.87 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – ML 49732	2.97 x 10 ⁵	3.15 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – PV 6380	2.00 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – PV 6380	1.94 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – PM 12453	1.87 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – PM 12453	2.01 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction

Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 1			
Antimicrobial Hemostatic Foam-54 – PA 9027	2.04 x 10 ⁵	6.10 x 10 ²	99.70
CONTROL – PA 9027	1.90 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – PA 15422	2.05 x 10 ⁵	7.00 x 10 ¹	99.97
CONTROL – PA 15422	2.02 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SC 10708	1.85 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SC 10708	1.70 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SM 8100	1.58 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SM 8100	1.98 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SM 14756	2.15 x 10 ⁵	1.60 x 10 ²	99.93
CONTROL – SM 14756	1.82 x 10 ⁵	> 3.00 x 10 ⁷	No Reduction
Antimicrobial Hemostatic Foam-54 – SA 6538	2.12 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SA 6538	2.01 x 10 ⁵	3.90 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – SA 25923	2.31 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SA 25923	2.14 x 10 ⁵	3.70 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – SM 35668	2.06 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SM 35668	1.97 x 10 ⁵	2.14 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – CA 10231	2.90 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – CA 10231	2.35 x 10 ⁵	3.50 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – AN 6275	2.05 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – AN 6275	1.75 x 10 ⁵	3.15 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – AN 16404	2.40 x 10 ⁵	2.10 x 10 ²	99.91
CONTROL – AN 16404	2.50 x 10 ⁵	3.40 x 10 ⁵	No Reduction

Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 2			
Antimicrobial Hemostatic Foam-54 – SM 35668	2.47 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – SM 35668	2.66 x 10 ⁵	3.40 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – CA 10231	2.20 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – CA 10231	2.50 x 10 ⁵	3.90 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – AN 6275	2.50 x 10 ⁵	< 1.00 x 10 ¹	> 99.99
CONTROL – AN 6275	2.70 x 10 ⁵	3.00 x 10 ⁵	No Reduction
Antimicrobial Hemostatic Foam-54 – AN 16404	2.65 x 10 ⁵	5.50 x 10 ¹	99.98
CONTROL – AN 16404	2.75 x 10 ⁵	3.20 x 10 ⁵	No Reduction

Sample Identification	Organism Count (CFU/ml)		Percent Reduction
	Zero Time	24 Hour	
DAY 3			
Antimicrobial Hemostatic Foam-54 – AB 19606	1.73×10^5	1.60×10^2	99.91
CONTROL – AB 19606	2.25×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – BA 9372	2.02×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – BA 9372	2.06×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – BD 19416	2.78×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – BD 19416	2.84×10^5	2.46×10^6	No Reduction
Antimicrobial Hemostatic Foam-54 – CD 13812	2.05×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – CD 13812	2.33×10^5	2.35×10^5	No Reduction
Antimicrobial Hemostatic Foam-54 – VRE 51575	1.69×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – VRE 51575	1.89×10^5	4.65×10^6	No Reduction
Antimicrobial Hemostatic Foam-54 – EC 8739	2.40×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – EC 8739	2.51×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – EC 25922	2.14×10^5	5.00×10^1	99.98
CONTROL – EC 25922	2.05×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – KP 4352	1.62×10^5	8.65×10^2	99.47
CONTROL – KP 4352	1.90×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – ML 49732	1.95×10^5	1.50×10^1	99.99
CONTROL – ML 49732	2.12×10^5	3.90×10^5	No Reduction
Antimicrobial Hemostatic Foam-54 – PV 6380	1.84×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – PV 6380	1.84×10^5	$> 3.00 \times 10^7$	No Reduction
Antimicrobial Hemostatic Foam-54 – PM 12453	1.74×10^5	$< 1.00 \times 10^1$	> 99.99
CONTROL – PM 12453	1.79×10^5	$> 3.00 \times 10^7$	No Reduction

Appendix D:

1. Introduction

Purpose

The test article identified below was extracted and the extract was subjected to an *in vitro* cytotoxicity study to determine whether leachables extracted from the material would cause cytotoxicity.

Testing Guidelines

The testing procedures are based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods.

Dates

Test Article Receipt: December 2, 2008
Cells Dosed Date: December 24, 2008
Observations Concluded Date: December 26, 2008

GLP Compliance

The study initiated by protocol signature on December 16, 2008, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

2. Materials

The test article provided by the sponsor was identified and handled as follows:

Test Article Name: Antimicrobial Hemostatic Foam (Max Load)

Test Article Identification: Code: 54-NOV-08

Stability Testing: In progress (per sponsor)

Expiration Date: Stable for duration of intended testing (per sponsor)

Strength, Purity and Composition:
Strength: 54 g of Silver glass particles in 100% Polyurethane foam
Purity: Pure Silver glass particles
100% PU foam; Composition: 54 g AG Glass in 150 g PU foam

Physical Description of the Test Article: White polyurethane foam with silver & glass particles

Storage Conditions: Room Temperature

Extraction Vehicle: Single strength Minimum Essential Medium supplemented with 5% fetal bovine serum and 2% antibiotics (10 units/mL penicillin, 10 µg/mL streptomycin and 2.5 UG/mL amphotericin B) designated as 1X MEM.

Negative Control Article: High density polyethylene (HDPE)

Negative Control Article Stability Testing: Marketed product stability characterized by its labeling.

Negative Control Article Strength, Purity and Composition:
HDPE: Strength: Not applicable, no active components in the formulation; Purity: Meets USP <661> Polyethylene Containers, Multiple Internal Reflectance, Thermal Analysis, Heavy Metals, and Non-Volatile Residue; Composition: Neat CAS #: 9002-88-4.

Reagent Control Article: Single strength Minimum essential Medium (1X MEM)

Summary

This *in vitro* study was conducted to evaluate Antimicrobial Hemostatic Foam (Max Load), Code: 54-NOV-08, for potential cytotoxic effects following the guidelines of International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods. A single preparation of the test article was extracted in Minimum Essential Medium supplemented with 5% serum and 2% antibiotics at approximately 37°C for a minimum of 24 hours. The negative control, reagent control, and positive control were similarly prepared. Triplicate monolayers of mouse fibroblast cells (L-929) were dosed with each extract and incubated at approximately 37°C in presence of 5% CO₂ for approximately 48 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

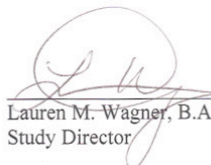
Under the conditions of this study, the test extract showed evidence of causing slight to mild cell lysis and toxicity. The test article met the requirements of the test since the grade was equal to a grade 2 or less (slight to mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

Supervisory

Personnel:

Don R. Pohl, B.S.
Christina L. Ovall, B.A.
Todd A. Festerling, B.S., M.S.

Approved by:


Lauren M. Wagner, B.A., M.S., ALAT
Study Director

1-6-09
Date Completed

Authorization for duplication of this report, except in whole, is reserved pending NAMSA's written approval.

GLP REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Vinesh Naik
Noble Biomaterials, Inc.
300 Palm Street
Scranton, PA 18505

CONFIDENTIAL

STUDY TITLE

Cytotoxicity Study Using the ISO Elution Method

TEST ARTICLE NAME

Antimicrobial Hemostatic Foam (Max Load)

TEST ARTICLE IDENTIFICATION

Code: 54-NOV-08

NAMSA

PEOPLE > SCIENCE > SOLUTIONS

P.O. Number
P81420

Lab Number
08T_56902_02

V0014_130
GLP Report

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4. Method

Triplicate culture wells were selected which contained a sub-confluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 2 mL of the test extract in each well. Similarly, the growth medium in triplicate 10 cm² wells was replaced with 2 mL of the reagent control, negative control and the positive control. The wells were incubated at approximately 37°C in 5% CO₂ for approximately 48 hours.

Following incubation, the cultures were examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

The color of the test medium was observed to determine any change in pH. A color shift toward yellow indicates an acidic pH range and a color shift toward magenta to purple indicates an alkaline pH range.

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. The test sample met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

5. Results

Mild cytotoxicity was noted. No pH shift observed at approximately 48 hours. The reagent control, negative control and the positive control performed as anticipated. The individual reactivity grades are shown in Appendix 1.

6. Conclusion

Under the conditions of this study, the test extract showed evidence of causing slight to mild cell lysis and toxicity. The test article met the requirements of the test since the grade was equal to a grade 2 or less (slight to mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

7. Quality Assurance

Inspections were conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report was reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities was issued with the report.

8. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files.

Appendix 1 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (A)	30	20	30	2	Mild
Test (B)	10	20	20	1	Slight
Test (C)	10	20	20	1	Slight
Negative Control (A)	0	0	0	0	None
Negative Control (B)	0	0	0	0	None
Negative Control (C)	0	0	0	0	None
Reagent Control (A)	0	0	0	0	None
Reagent Control (B)	0	0	0	0	None
Reagent Control (C)	0	0	0	0	None
Positive Control (A)	100	100	100	4	Severe
Positive Control (B)	100	100	100	4	Severe
Positive Control (C)	100	100	100	4	Severe

Note: A, B and C denote replicates.

Appendix E:

Silver Foam Hemostatic Bandage as an Effective Hemostatic and
Antimicrobial Agent in the Treatment of Traumatic Wounds Requiring
Secondary / Delayed Primary Closure

Michael F. Moore, MD
Wound Institute & Research Center
Noble Biomaterials

FY 2008

Silver Foam Hemostatic Bandage as an Effective Hemostatic and
Antimicrobial Agent in the Treatment of Traumatic Wounds Requiring
Secondary / Delayed Primary Closure

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Silver Foam Hemostatic Bandage as an Effective Hemostatic and
Antimicrobial Agent in the Treatment of Traumatic Wounds Requiring
Secondary / Delayed Primary Closure

PROTOCOL TEAM ROSTER

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**Silver Foam Hemostatic Bandage as an Effective Hemostatic and
Antimicrobial Agent in the Treatment of Traumatic Wounds Requiring
Secondary / Delayed Primary Closure**

Sponsor Information and Relationships

**This study is being sponsored by the Department of Defense (DOD) and the
Telemedicine and Advanced Technology Research Center (TARTC)**

Noble Biomaterials is a privately owned company engaged in the development of silver metalized products to treat and prevent antimicrobial infections. Dr. Michael F. Moore, MD is the medical director of the Wound Institute & Research Center (FWA 00013018; Scranton Temple Residency Program IRB) and acts as the medical consultant for Noble Biomaterials to oversee and direct medical research in the applications of these products. The Wound Institute & Research Center is also engaged in the clinical evaluation of specific bandage products. Noble Biomaterials has no financial interest or governing role in the Wound Institute & Research Center. The Wound Institute and Research Center will not act as a study site for this research project.

Community Medical Center Hospital (FWA 00010261; Scranton Temple Residency Program IRB) is a non-profit hospital in Scranton, PA with a level II trauma center, which will participate, in the clinical evaluation of the antimicrobial hemostatic bandage. Michael F. Moore, MD is on active staff at Community Medical Center Hospital and will act as the principal investigator for this site. Dr. Moore and the Wound Institute and Research Center have no financial interest in Community Medical Center Hospital.

Albany Medical Center Hospital (FWA 00001314; Albany Medical Center IRB) is a non-profit tertiary care center with a level I trauma services and will participate in the clinical evaluation of the antimicrobial hemostatic bandage. Carl Rosati, MD is a general surgeon employed by the Albany Medical Center Hospitals and acts as the director of the trauma services. He will act as the principal investigator for this site.

INTRODUCTION

1.1 Background and Prior Research

In evaluating traumatic injuries, the surgeon has to decide if closing an injury primarily is feasible or will be successful. Many factors enter into this decision. The extent of trauma, the extent of contamination, the time interval between injury and medical attention, and the degree of other associated injuries are some of the factors that determine if primary closure is an option.¹ If primary closure is not an option, delayed primary closure or closure by secondary intention are the two disciplines that allow for treatment of the injury. After debridement of the wound of any non-viable tissue and achieving hemostasis, the surgeon must apply a bandage that will continue to absorb exudates, mitigate against infection, prevent further injury, and aid in maintaining hemostasis. Traditionally this bandage has been gauze of varying sizes and construct.² Though it has functioned over decades, gauze has limited absorbent capacity, requiring frequent changes. Its hemostatic effect is based on pressure and the provision of a scaffold for blood clots to form. Though initially sterile, gauze provides no other antimicrobial effect and is easily and rapidly colonized by bacteria.

The frequent dressing changes that gauze requires are problematic due to the significant pain the procedure induces, sometimes requiring general anesthesia, the risk of blood loss, the increased time spent by health care providers addressing the needs of the patients and the increased resources allocated to the maintenance of the dressings.

Noble Biomaterials previous work has led to the incorporation of silver into various medical dressings. In conjunction with Johnson and Johnson the production of an antimicrobial alginate dressing was developed for moderately exudating wounds and is presently used in this clinical arena. The development of hydrophobic antimicrobial foam with Kinetics Concepts Incorporated (KCI) also produced another medical dressing exhibiting antimicrobial activity, which is widely used for secondary closure in known infected wounds. The use of a metalized packing strip in facilitating the treatment of chronic osteomyelitis was also developed by Noble Biomaterials.³ The success of incorporating the antimicrobial effect of silver into varying adsorptive bandage types became the nidus for the research into the feasibility maintaining the antimicrobial effect, increasing the absorptive capabilities and incorporating a hemostatic agent.

The silver foam hemostatic bandage designed by Noble Biomaterials and the Wound Institute addresses many of the issues that face the surgeon in dealing with secondary or delayed primary closure. The increased adsorptive capacity of the foam allows fewer dressing changes and acts as a lattice for activation of the intrinsic cascade. The increased adsorptive properties also mitigate the need for drains, as exudates are drawn into and contained within the hydrophilic foam. The hemostatic agent activates the intrinsic cascade, facilitating hemostasis. The broad-spectrum antimicrobial effect of silver deters against bacterial colonization and infection.

Purpose:

The purpose of this research is to evaluate the effectiveness of the silver foam hemostatic bandage in reducing morbidity and mortality of secondary surgical infection, reducing the need for blood products, reducing the number of dressing changes, evaluating the safety of the bandage and reducing the time interval from injury to definitive surgical repair.

1.2 Rationale:

The rationale behind this research is that an effective antimicrobial hemostatic bandage would decrease the frequency of dressing changes. This decrease in the number of dressing changes would translate into decreased nursing time allocation, decreased patient discomfort, decrease blood product usage, and decreased analgesic requirements. It would allow the dressing to stay in place for a longer period of time while mitigating against local and systemic infection. It would also allow for the transportation of patients more efficiently by eliminating the need to have dressing products accompany the patient in transit.

2 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

- i. To evaluate the blood product requirements during use of the silver foam hemostatic bandage compared to standard gauze dressings.
- ii. To evaluate the frequency of silver foam hemostatic dressing changes compared to the frequency of standard gauze dressing changes throughout the course of treatment.
- iii. To evaluate the time interval required before initiation of a definitive surgical procedure allowing closure of the wound.

2.2 Secondary Objectives

- i. To develop definitive protocols defining the proper use and application of a antimicrobial hemostatic dressing
- ii. To evaluate the antimicrobial effect of silver.

2.3 Study Design

Sterile Silver Hemostatic Bandages developed by Noble Biomaterials will be delivered to the trauma centers. The bandages developed by Noble Biomaterials will be a composite of hydrophilic foam and silver metalized glass beads. The foam will have a pore size of fifty pores per square inch and have a silver weight content of 20%. The bandages will be supplied in three sizes and two thicknesses. The first size bandage is four inches in length and width and will have a thickness of one quarter of an inch. The second size bandage will be five inches in width and nine inches in length and will have a thickness of one half inch. The third size bandage will be two inches in width and length and one quarter inch in thickness. All three bandage sizes will have the same pore size and silver content. Silver Hemostatic dressings would be provided to the civilian trauma centers for the treatment of wounds that, because of their nature would preclude the surgical option of primary closure.

The patients would follow existing trauma protocols established at each center dictating stabilization and resuscitation. Surgical intervention would be based on individual patient presentation and at the discretion of the trauma physician. The surgeon treating the patient will determine whether the wound should be treated by the use of delayed healing parameters. Delayed healing parameters would include those wounds of the trunk, chest, extremity, abdomen or head where (1) the tissue deficit could not be closed without tension (2) the extent of soft tissue injury could not be accurately assessed (3) the amount of foreign body contamination precluded adequate debridement. The selection of the bandage size would be based on the size of the tissue deficit compared to the dressing measurements.

Once it has been determined and permission has been obtained for the patient to participate in the study the bandage selection will be based on the last number in the year that the patient was born with odd numbers being assigned to the gauze arm and even numbers being assigned to the foam arm of the study. An independent and separate study number will be assigned to the chart to identify and track the study participants. These numbers will be in sequential ascending order based on the time of entry into the study. The numbers will be entered in a three digit format and be preceded by a letter code of either G or F. The patient will then be treated with the selected bandage until such time as a definitive final procedure is performed or the patient conditions dictate that healing by secondary intent will be employed. The end point of the study will be when either of these two clinical decisions is made.

The wounds will be categorized based on anatomical location, surface area, and whether gauze or foam dressings were employed.

The duration of the study will begin when the selected bandage was employed in the treatment of the patient. The end point for the assessment of data will be either closure by secondary intention or that definitive surgical closure was employed.

The primary objectives will be obtained by the following methods:

The antimicrobial effect will be assessed by monthly random selection of bandage lots to obtain samples for bacterial testing. Using the trauma centers bacteriologic finger print, selective organism will be plated and tested against the antimicrobial effect of the foam samples as to their ability to maintain a seventy-two-hour log reduction of colony forming units. This information will be collected by the data entry personnel using the PMO1 form (see Appendix A) in the data base computer.

Blood product usage will be entered at the end of the patient's participation in the study by the data entry personnel. The data will be obtained from the medical record. The data will be entered on the PMO2 form (see Appendix A) in the data base computer as to the type of product and date of administration and date of the last bandage change. Analysis of this data will be undertaken to evaluate blood product usage as it compares to dressing type and time of dressing change.

The date and type of dressing change will be entered on the PMO3 form (see Appendix A) in the data base computer by data entry personnel. The data will be obtained from the medical record at the end of the study period.

The time of the treatment period will be defined as when the initial dressing was applied and when the treatment regime either change or a definitive surgical procedure was employed to close the wound. The time interval will be obtained from the medical record by the data entry personnel and entered into the computer data base on form PMO4 (see Appendix A).

2.4 Roles and Responsibilities:

Nanci P. Dobson, RN, Clinical Research Coordinator:

The primary responsibility of the Clinical Research Coordinator will be to educate the nursing personnel in the proper use and application of the bandages, to review and assure that the bandages were placed in compliance with the study design protocol as it applies to nursing duties, to review the outcomes and opinions of the nursing staff as it applies to the implementation of the bandage from a nursing perspective, to assure the consistency of compliance of the nursing staff within the multi site trauma centers, and to aid in evaluating the data analysis as it pertains to nursing duties.

Michael F. Moore, MD, FACS, Noble Biomaterials and the Wound Institute and Research Center; Grant Principal Investigator:

The responsibility of the Principal Investigator for Noble Biomaterials and the Wound Institute and Research Center will be to oversee the manufacturing and delivery of the bandages to the multi trial sites, to review and delineate the study protocol with the participating surgeons, to assess by regular meetings with the participating surgeons the progression of the study as it relates to clinical outcomes, to assure the consistency of compliance of the participating surgeons within the

multi site trauma centers, to tabulate the clinical findings and review these findings for statistical significance with the designated statistician, to review these findings with the participating trauma centers as it relates to protocol criteria and any changes that might be evident as the result of this study, to review the clinical findings with Noble Biomaterials as it pertains to bandage production and market potentials, to coordinate the medical publication of this data.

Individual Investigator Trauma Center

The responsibility of the Individual Investigator at the trauma center will be to oversee the compliance of the protocol with the participating surgeons, to monitor the existing clinical trauma protocols with compliance to the study protocols, to communicate with the principal investigator compliance with the clinical progression of the study, to monitor for any adverse effect emanating from the study, to appoint an independent medical monitor who is qualified to oversee and provide medical care to research volunteers for conditions that may arise from the study.

Medical Monitor at Trauma Center:

The responsibility of the medical monitor will be to monitor the research subjects enrolled in the study for any unforeseen medical conditions that might arise and to treat and report these conditions to the principal investigators. The medical monitor will review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor will comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor will also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the ORP HRPO.

Data Entry Personnel at Trauma Centers

The responsibility of the data entry personnel will be to extract from the medical record as it relates to the study protocol and enter this data into the data base for statistical analysis.

3.0 STUDY POPULATION

The study population would be trauma patients admitted to a trauma center and whose injuries prevented definitive primary closure. Based on the incidence of injuries in the United States per 100,000 and using a 95% confidence level and a confidence interval of 6% it is calculated that the sample size would have to included a study population of 300 patients.

3.1 Inclusion Criteria

- a. Any open wound of the trunk, chest, or extremity that could not be closed primarily.
- b. A surgical incision dehiscent
- c. Infected wounds requiring open treatment
- d. Open Fractures

3.2 Exclusion Criteria

- a. Allergy to silver by history or by clinical manifestation during the study period.
- b. Allergy to the selected hemostatic agent by history or by clinical manifestation during the study period.
- c. Allergy to polyethylene foam by history or clinical manifestation during the study period.
- d. Pregnant female subjects, as determined by urine test.
- e. Any patient under the age of eighteen.

3.3 Recruitment Process

Patients admitted to the Community Medical trauma service or the Albany Medical Center trauma service and requiring operative intervention will be candidates for enrollment into the study. Such patients will be referred to the principal investigators conducting the study. The principal investigator would then evaluate the patient as to the eligibility to participate in the study. If the patient is an appropriate candidate as determined by the principal investigator, the antimicrobial protocol would be presented to the patient for his or her consideration. The depth and scope of the study would be explained to the patient by the principal investigator. The principal investigator would also informed the patient what the goals of the study where and what risks and benefits are associated with the study. Once the study has been presented to the patient by the principal investigator, the permission to participate will be given to the patient twenty four hours prior to any planned surgical intervention. If the patient agrees to participate in the study the principal investigator will obtain and sign the permission to participate in the study

The design study and protocol criteria will be presented and reviewed with the clinical principal investigators at the designated trauma centers prior to the initiation of the study. Prior to the initiation of the study the protocol will be presented to the trauma center service surgical staff at the regular staff meeting to enlist their

participation. The inclusion and exclusion criteria will be reviewed as it relates to previous trauma center admissions as to the historical appropriateness of this study. Weekly review of the patients enrolled into the study will be undertaken between the grant principal investigator and the clinical principal at the trauma center to ensure ongoing participation and recruitment. Pregnant female subjects, as determined by universal urine screening tests will be excluded from the study.

3.5 Participant Retention

The patient will remain in the study until such times that a definitive surgical procedure is performed to close the wound. Patients will be also retained in the study until such time that closure by secondary intent has been selected to treat the wound. In those patients selected to have their wounds healed by secondary intention, the retention period will extend to closure of the wound occurs.

3.6 Participant Withdrawal

Patients will be withdrawn from the study if they exhibit any allergic reactions to the bandage. Withdrawal from the study will also occur if the patient wishes to be removed from the study.

4 STUDY TREATMENT/PRODUCTS/INTERVENTION

4.1 Treatment /Product/Intervention Formulation/Content

The bandage formulation is composed of medical grade hydrophilic foam with a pore size of fifty pores per square inch and containing twenty six per cent silver. The bandages will come in three sizes. The four by four bandage will be four inches in width and length and be one quarter inch in thickness. The two by two will be two inches in width and length and one quarter inch in thickness. The eight by ten bandage will be eight inches in width, ten inches length and one half inch in thickness. The bandages will be individually packaged and will have been sterilized by gamma radiation.

4.2 Treatment /Product/Intervention Regimen

The selection of which hemostatic bandage will be at the discretion of the operating surgeon and based on his or her clinical assessment of the patient's physiologic need. Once selected the protocol as stated in the study procedures will be employed.

4.3 Treatment /Product/Intervention Administration

Noble Biomaterial will be responsible for delivering to the clinical testing sites the sterilized antimicrobial hemostatic hydrophilic foam dressings. The dressing allocation

will be based on the review of existing patient demographics based on CPT diagnoses. Ongoing monitoring of the diagnosis and treatment requirements will be made to assure adequate bandage inventory.

4.4 Treatment/Product/Intervention Supply and Accountability

As outlined in section 4.3 the bandage allocation will be based on clinical need. Serial numbering of bandage lots will be recorded and monitored based at the clinical testing sites and correlated with clinical application. Adjustments as to ongoing need will be assessed on clinical need and use. Bandages that are not used will be returned to Noble biomaterials.

4.5 Adherence Assessment

Ongoing assessment by the treating surgeon, the principal investigator and Noble Biomaterials will be undertaken to assure compliance with protocol criteria. If a treatment option has to be changed because of clinical judgment, patient request or allergic reaction, conversion to standard gauze dressings will be initiated.

4.6 Toxicity Management

If in the clinical judgment of the treating surgeon there is a clinical toxic manifestation either locally or systemically to the bandage protocol, cessation of the treatment regime will be enacted.

4.7 Concomitant Medication

Enrolled study participants will be allowed to continue any and all concomitant medications. It is anticipated that there will be no medication that would not be permitted in this study.

5 STUDY PROCEDURES

5.1 Bandage Selection:

The selection of size and amount of dressing required will be made by the operating surgeon based on his/her clinical judgment. Prior randomization as to the type of bandage would have been performed. Continued use of the same type of bandage would be maintained until definitive surgical intervention was accomplished.

5.2 Data collection

Specific data as to the type and size of the dressing, date of the dressing change, the type and amount of any blood product used at the time of the dressing change, the time interval for surgical intervention and the total number of days and number of dressing changes until closure by secondary intent occurs will be collected by study team members at both research sites. This information will then be collected by designated data entry personnel and entered into the computer data base using the PMO1, PMO2, PMO3, and PMO4 forms⁴ PMO1 will be used to assess the bactericidal effect of the bandages. PMO2 will be employed to collect information on the blood product usage. PMO3 will be used to track dressing information and PMO4 will be employed to evaluate the time to definitive surgical intervention.. The data will be collected on a weekly basis and stored in a secured password and encrypted HIPAA compliant computer at both trauma facilities. The data will be entered using a number assignment based on the time of entry into the study, the date of entry and the arm of the study to which the patient was assigned. For example, the number 140070909GA would indicate that the patient was entered into the gauze arm of the study at 1400 hours on the ninth of July 2009 at Albany Medical Center Trauma Service. No identifying patient information will become part of the data base.

The study sites will be responsible for the collection and entry of the data, which data will be transmitted to the Wound Institute and Research Center for analysis. Transmission of this data will be done electronically using a one hundred twenty eight encryption key.

5.3 Data analysis

The data will be analyzed for statistical relevance using multi varied analysis to evaluate the efficacy of the metallized hemostatic bandage to achieve its stated objectives. The review will occur on a weekly basis. Review will encompass the number of patients entered into the study, those patients that have completed the study, review of data entered as to accuracy and completeness, review of bandage use and compliance, review of adverse events and assessment as to the completion of the study.

Publication of the data based on objective findings will be made.

6 SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 SAFETY MONITORING

Patients participating in this study will be enrolled in existing protocols for trauma related injuries. Those existing safety monitors will be employed by the participating trauma centers.

6.2 Adverse Event Reporting Requirements

A single occurrence of a serious, unexpected event that is uncommon and strongly associated with the use of the bandage

A single occurrence, or more often a small number of occurrences, of a serious, unexpected event that is not commonly associated with the use of the bandage, but uncommon in the study population

Multiple occurrences of an AE that, based on an aggregate analysis, is determined to be an unanticipated problem.

An AE that is described or addressed in the investigator's brochure, protocol, or informed consent documents, but occurs at a specificity or severity that is inconsistent with prior observations.

Any other AE or safety finding (e.g., based on animal or epidemiologic data) that would cause the sponsor to modify the investigator's brochure, study protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects.

The period which AE must be reported will be defined as the initiation of the study and thirty day following discharge from the hospital.

6.3 Serious Adverse Event Reporting Requirements

A serious AE that is described or addressed in the investigator's brochure, protocol, or informed consent documents, but for which the rate of occurrence in the study represents a clinically significant increase in the expected rate of occurrence

A serious adverse event (SAE) is any AE that is

- Fatal
- Life Threatening
- Requires or prolongs hospital stay
- A congenital anomaly or birth defect
- An important medical event

Any SAE will be reported within twenty four hours of its occurrence.

Any SAE will be reported to the Principal Investigator:

Michael F. Moore, MD

Phone: (570) 961-8000

Fax: (570) 961-8007

7 STATISTICAL CONSIDERATIONS

7.1 Review of Study Design

The study is designed to evaluate the effect of an antimicrobial hemostatic bandage

7.2 Endpoints

7.2.1 Primary Endpoints

- The blood product requirements following dressing changes with foam as compared to gauze.
- The frequency of dressing changes of foam as compared to gauze.
- The time interval from initial dressing to definitive surgical intervention of foam as compared to gauze.

7.2.2 Secondary Endpoints

- The protocol that delineates the most effective way of implementing the antimicrobial hemostatic bandage.
- The antimicrobial effect of silver.

7.3 Accrual, Follow-up and Sample Size

7.4 Random Assignment

The selection of whether the foam bandage or gauze bandage will be determined by the random selection of a foam or gauze envelope which will have been previously divided into equal quantities and distributed to the trauma centers. The initial allocation will be employed until all the bandages in the initial allocation have been used to insure equal utilization. Any further bandage use will be distributed using the same criteria. The size of the wound will determine which size bandage is employed as will the opinion of the operating surgeon.

7.5 Blinding

Data regarding the primary objectives and outcomes of the study will be collected by the data entry personnel.. Though individual identifiable patient data will be known to the surgical and nursing staff, analysis and comparison of individual identifiable patient data will not be recorded or available to the study team members. Review of this data on a weekly basis with the individual investigators will be undertaken to

assess any immediate benefit of one type of dressing over the other. Any immediate or inherent benefit of one study arm would result in that finding or clinical outcome to be unblinded and presented to the study group for consideration.

7.6 Data Analysis

7.7.1 Primary Analyses

Paired t test analysis.

7.7.2 Secondary Analyses

Not Applicable

8 HUMAN SUBJECTS CONSIDERATIONS

8.1 Ethical Review

This study is to be conducted according to US and international standards of GCP (FDA) Title part 312 and International conferences of Harmonization guidelines, applicable government regulations, and institutional research policies and procedures. This protocol and any amendments will be submitted to a properly constituted Review Board or Ethics Committee (EC) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision to the principal investigator prior to the commencement of this study.

Subsequent to initial review and approval, the responsible IRBs/ECs will review the protocol at least annually. The Investigator will make safety and progress reports to the IRBs/ECs at least annually, and within three months of study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

8.2 Informed Consent

If it is anticipated that in the initial assessment of the patient that delayed primary closure of the injury is a possible treatment option then the informed consent for the antimicrobial hemostatic bandage will be presented to the patient for consideration.. Only after the patient has agreed to enroll in the study and has signed an informed consent will the patient be assigned a PIN based on the criteria described in the study

design.. No patient will be enrolled in the study unless an informed consent has been obtained and witnessed by the principal investigator

8.3 Risks

The risks of the study would be a local or systemic allergic reaction to silver, a local or systemic allergic reaction to the polyethylene foam and a local or systemic reaction to the glass beads metalized with silver. There is also the risk that the bandage would be ineffective in controlling bacterial growth and that a local or systemic infection could develop.

8.4 Benefits

If upon completion of the study it is found out that the antimicrobial hemostatic bandage has successfully diminished blood requirements, diminished the use of antimicrobial agents and has diminished the hospital length of stay, then society would benefit. The benefits society would see decreases in allergic reactions secondary to the administration of blood products and antimicrobials, decrease in the expenses associated with dispensing such products. The decrease in hospital length of stay would allow the reallocation of the savings to other hospital programs. The success seen in the use of this bandage would allow its clinical use with first time responders and in the chronic wound care arena.

8.5 Incentives/Compensation

Neither incentives nor remuneration is anticipated for the participants of this study.

8.6 Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in password protected encrypted electronic medical record (EMR) with access limited to study staff. All local databases will be secured with password-protected access systems.

8.8 Study Discontinuation

The study also may be discontinued at any time by the US Food and Drug Administration, other government or regulatory authorities, and/or site IRBs/ECs.

9 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Local Laboratory Specimens

No laboratory specimens will be collected as part of this study.

9.2 Central Laboratory Specimens

Not Applicable

9.3 Biohazard Containment

Each Trauma site will follow standard existing protocols for the containment and disposal of biohazard materials.

10 ADMINISTRATIVE PROCEDURES

10.1 Study Activation

Study Activation will begin with the approval of the IRB board governing the selected trauma sites, and after review and approval by the USAMRMC Office of Research Protections Human Research Protections Office (ORP HRPO).

10.2 Study Coordination

Coordination of the study will be performed by the Wound Institute and Noble Biomaterials. Noble Biomaterials will ensure the acquisition and delivery of the

antimicrobial hemostatic bandage to both research sites. The Wound Institute will be responsible for the acquisition of the data, entry of the data, and statistical analysis of the data. The data entry will be governed by the use of standardized forms (see Appendix A) developed for this study so as to achieve consistency. Coordination of the study will be accomplished by the clinical coordinator through the use of weekly and monthly meetings.

10.3 Study Monitoring

Study monitors from the Wound Institute and Research Center will visit the site to

- verify compliance with human subjects and other research regulations and guidelines;
- assess adherence to the study protocol,
- confirm the quality and accuracy of information collected at the study site and entered into the study database.

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives US and in-country government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.4 PROTOCOL COMPLIANCE

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Principal Investigator. All protocol amendments must be submitted to and approved by the relevant IRB(s)/EC(s).

10.5 Investigator's Records

The individual investigator will maintain, and store in a secure manner, complete, accurate and current study records throughout the study. Study records include administrative documentation — including protocol registration documents and all reports and correspondence relating to the study — as well as documentation related to each participant screened for and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

10.6 USE OF INFORMATION AND PUBLICATIONS

Publication of the results of this study will be governed by existing protocols of the trauma centers and with the approval of Noble Biomaterials and the Wound Institute. Any publication or presentation regarding the outcome of this study will preclude the use of any patient information.

11 REFERENCES

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5. Gabay M. *Absorbable Hemostatic Agents*; Amer J Health-System Pharm 63(13):1255-1253 July 1, 2006
6. Wright J. et al: *Thermal Injury Resulting from Application of a Granular Mineral Hemostatic Agent*; The Journal of Trauma 57;2, Aug 2004